COMPARATIVE STUDIES ON RESPIRATION XVIII. RESPIRATION AND ANTAGONISM IN ELODEA

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Previous studies in this series have dealt with the relation between antagonism and respiration, but have not included tissues of higher plants containing normal amounts of chlorophyll.[1] The experiments here presented were designed to test the effects of mixtures of solutions of sodium and calcium chlorides on such tissues.

For this purpose the leafy stems of *Elodea canadensis* were selected. This has proven to be excellent material since it is hardy in respect to climatic conditions and laboratory manipulation while sufficiently sensitive to reagents. The leaves are exceedingly thin, and gaseous exchange is very rapid.

All the plants were collected from one place in a slowly flowing stream and, as long as it remained open, taken fresh at least once a week to the laboratory. The material used during the late winter and early spring was provided by a quantity of plants collected in December and kept in the greenhouse in large glass jars in a cool room. It thrived well and when tested gave normal results.

The method used for most of the experiments was that developed by Haas[2] in which the plants were immersed in solutions containing an indicator.[3] The production of CO_2 was measured by the change in color of the indicator. The standard buffer solutions containing the same indicator were mixtures of borax and boric acid.

The final experiments were carried out by the use of the apparatus described by Osterhout.[4] The curves closely resemble those obtained by the other method. The accuracy of measurement was greater. Since the two methods are alike in all but mechanical details, only the first will be discussed in full.

The procedure consisted in measuring the normal rate of production of CO_2 in tap water or distilled water and then testing the effect of a salt

[1] The experiments on wheat reported in a previous paper of this series were made upon germinating seeds which contained little or no chlorophyll. Cf. **Thomas, H. S.** Jour. Gen. Physiol. 1: 203. 1918.

[2] **Haas, A. R. C.** Science N. S. 44: 105. 1916.

[3] The plants were thoroughly washed to remove any adhering organisms. Microscopical inspection showed that the plants used for the experiments were almost free from bacteria.

[4] **Osterhout, W. J. V.** Jour. Gen. Physiol. 1: 17–22. 1918.

solution on the same material in the same tube. For normal respiration the material was placed in 10 cc. of water to which had been added 5 drops of a 0.01 percent solution of phenolsulphonphthalein. Both tap and distilled water were tried, and as no difference could be noted in the effects on the plant, distilled water was used exclusively, since the salt solutions were made up in it. This eliminated possible effects of the salts in the tap water.

Both the water and the salt solutions were brought to the proper alkalinity by the addition of a very dilute solution of sodium hydroxide, the same amount being added to each.

The plants selected were healthy stems, uniform in appearance, which averaged from 3 to 4 inches in length. These were kept in running water before use (to remove any excess of CO_2), and were then coiled and inserted in the tubes where the pressure of the coil held them in the middle of the tube. This kept them from interfering with the observation of the color of the solution (in the lower half of the tube) and with comparison with the color of a standard solution. The paraffined rubber tube at the top was then tightly clamped off after adding the water plus the indicator. A bubble of air, of uniform size in all experiments, was left below the clamp to aid in stirring the solution. The pH value of the water was brought to a little above 7.88, but it dropped to 7.88 shortly after the plants were placed in it. The exact time at which this point was reached was determined by matching its color with that of a buffer solution of pH 7.88 (which had the same concentration of indicator).

The contents of the tube were kept in constant motion by gentle stirring during the few minutes required for the evolution of enough CO_2 by the plant to change the color to match that of the second standard tube of pH 7.60.[5] This range of 7.88 to 7.60 was used in all experiments. In getting the normal rate, the amount of material used was adjusted to give a period of from 3 to 5 minutes in most cases.

That no acid other than carbonic was produced was shown by the fact that after the plant had changed the color of the indicator solution, it would rapidly return to the original color when a current of CO_2-free air was bubbled through it.

The normal period of respiration for each experiment was first determined. It was usually found that this period was practically constant for two hours or more, and if this was not the case the material was rejected. At least three readings (covering a period of at least 20 minutes) were taken, previous to the addition of the salt solutions, in order to establish the normal rate.

The temperature varied from 21 to 25° C. In the course of any one experiment, the temperature did not vary more than two degrees.

The solutions of salts were made up in large quantities and kept in

[5] The source of light was a "Daylight lamp." Cf. **Luckiesch.** Science n. ser. **42**: 764. 1915.

bottles of resistant glass. It was necessary to have them well stoppered, and to keep the bottles well filled in order to avoid absorption of carbon dioxide and the consequent reduction of alkalinity and increase of buffer action when made alkaline again.

Preliminary experiments showed that a concentration of 0.1 M sodium chloride was preferable for study. Anything above 0.2 M was found to give plasmolysis. A solution of 0.07 M of calcium chloride was taken as approximately isotonic with the 0.1 M sodium chloride, and all mixtures were made up with these concentrations.

In order to avoid a possible error by the buffer action of the solutions, these were tested by bringing the distilled water and all the solutions to an alkalinity of pH 8 and then adding a few drops of a solution of CO_2 in distilled water. All changed by approximately the same amount, which showed that there was no appreciable buffer action that would interfere in the measurements of production of CO_2.

The time curves of the production of CO_2 were plotted in the manner explained by Osterhout[6] and used in other papers in this series. Rate of respiration in percent is plotted against time in minutes, the normal rate (as determined before addition of salt) being taken as the reciprocal of the average period required to change the solution from PH 7.88 to 7.60; this rate was taken as 100 percent.

The behavior of the plant was not quite the same in the fall and spring seasons. In early spring, while working with material kept in the greenhouse, it was found that the solutions of pure salts and the mixtures were giving different rates of production of CO_2 than the same solutions had in the fall and winter. The shapes of the curves were not changed, but there was an increase in all the ordinates of from 5 to 15 percent. This prevented the inclusion of many data, for there were not enough experiments for certain points to give a complete curve by themselves. Nevertheless, they provide confirmation of the results presented; especially of the maximum points in figure 2.

Figure 1 shows the production of CO_2 in NaCl 0.1 M (curve A), in $CaCl_2$ 0.07 M (curve B), and in mixtures of these. It was found that all concentrations of NaCl (none above 0.2 M were tried) gave an increase,[7] while all those of $CaCl_2$ gave a decrease. In some of the weaker concentrations of $CaCl_2$ the rate showed a tendency to rise again after falling, but it remained below the normal. In low concentrations of NaCl the increase (of from 25 to 50 percent) lasted for at least 90 minutes, while in 0.1 M NaCl it fell off rapidly at first and then more slowly as shown in the typical curve.

A remarkable behavior was observed when the molecular proportions

[6] **Osterhout, W. J. V.** Jour. Gen. Physiol. 1: 171–179. 1918.

[7] B. Jacobi (Flora **86**: 289. 1889) states that NaCl 0.0496 M causes an increase in the production of CO_2 by Elodea, followed by a decrease.

were 98.62 NaCl to 1.38 $CaCl_2$; the rate increased and did not return to normal (as shown in curve D, figure 1).

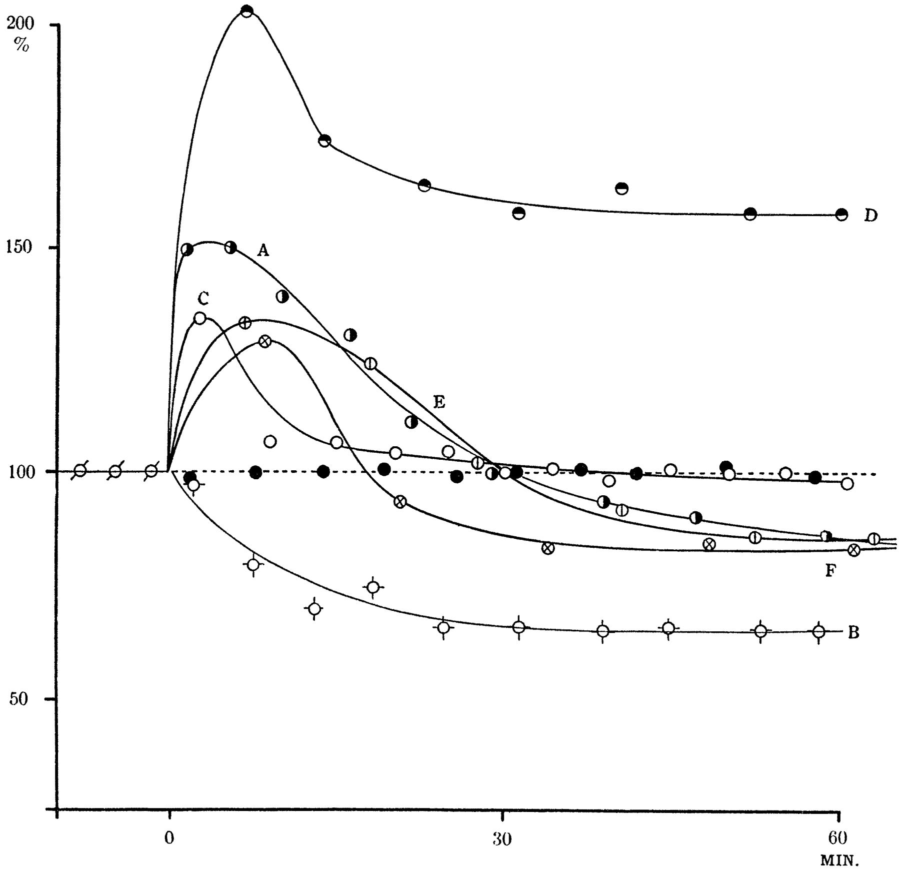

FIG. 1. Curves showing the effects of NaCl and $CaCl_2$ on the respiration of *Elodea canadensis*. The horizontal line at the left of the point marked O on the abscissae represents the normal rate of respiration before the addition of the salt.

Curve A represents the rate of respiration in NaCl 0.1 M, curve B in $CaCl_2$ 0.07 M; the other curves represent the rate in mixtures of these having the following molecular percentages: curve C in 99.65 percent NaCl+0.35 percent $CaCl_2$; curve D in 98.62 NaCl +1.38 $CaCl_2$; curve E in 98.85 NaCl+1.15 $CaCl_2$; curve F in 98.28 NaCl+1.72 $CaCl_2$. The broken line represents the control in distilled water. Each curve represents a typical experiment.

The determination of antagonism should preferably be made at a period of the experiment when both solutions of pure salts give either an increase or a decrease in rate. Since the former was impossible, an exposure of one hour was chosen, at which period both gave a decrease and the time curves had become nearly horizontal. Figure 2 shows the rates of respiration in various mixtures and in the solutions of pure salts. The ordinates represent

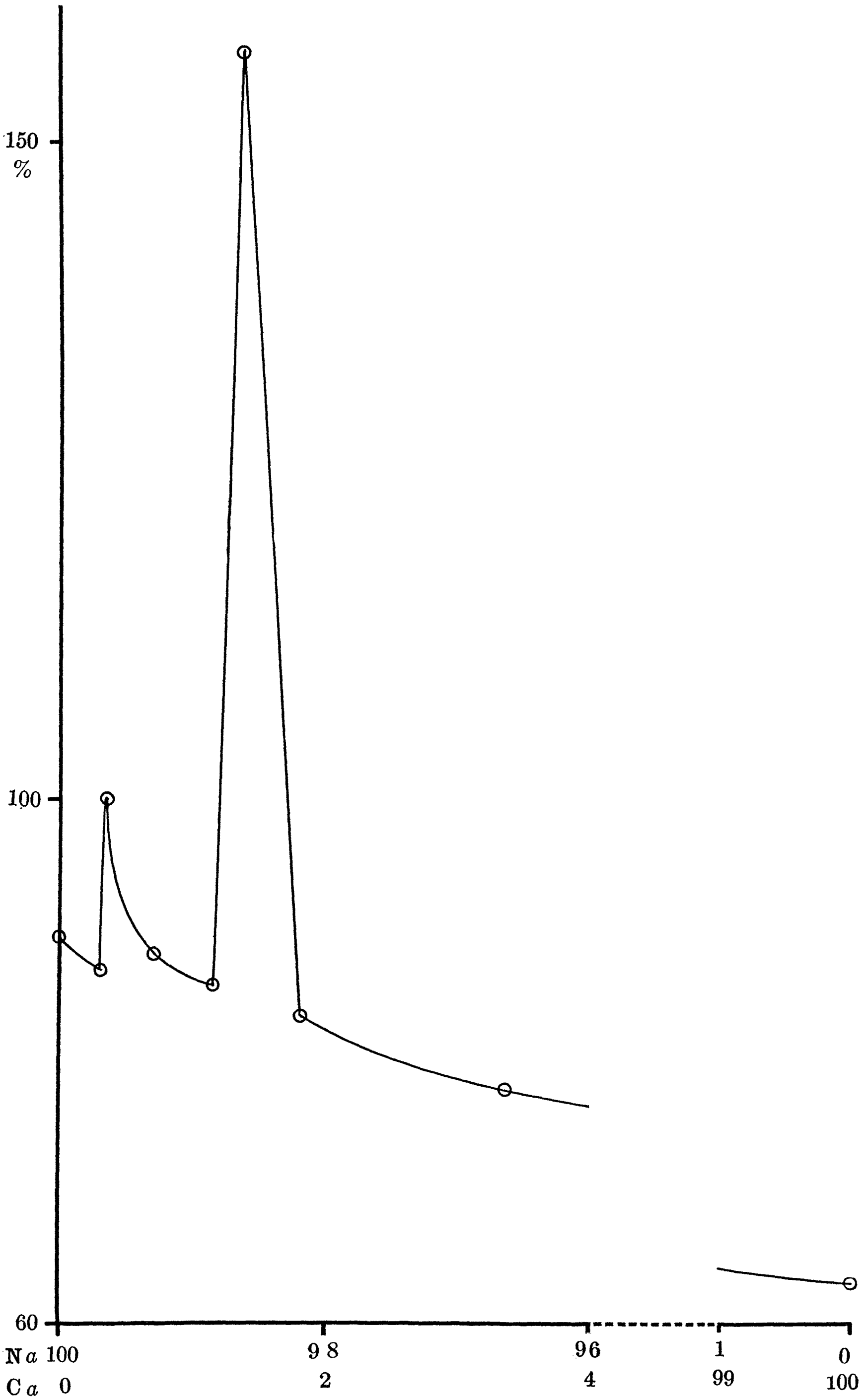

FIG. 2. Antagonism curve showing the effect of NaCl 0.1 M+$CaCl_2$ 0.07 M and of mixtures of these upon the respiration of *Elodea canadensis* after an exposure of one hour. The abscissae represent molecular proportions. Each point represents the average of 3 or more experiments; probable error of the mean, less than 4 percent of the mean (except in one case where it amounts to less than 6 percent).

the rate of production of CO_2 after the plants had been in the solutions for one hour.

The figure shows that while the molecular proportions 99.65 NaCl to 0.35 of $CaCl_2$ give normal respiration, a decrease occurs at other proportions except that of 98.62 to 1.38 of $CaCl_2$. This form of the antagonism curve of NaCl vs. $CaCl_2$ is unique, as is evident on comparing it with others in this series as well as with those in which growth, length of life, etc., are used as criteria.[8] In order to explain this peculiar effect additional experiments will be necessary, and further discussion is deferred until these can be carried out.

Summary

1. Solutions of NaCl cause an increase in respiration, which is followed by a decrease, while solutions of $CaCl_2$ cause only a decrease.

2. After a sufficient length of exposure both NaCl and $CaCl_2$ depress the respiration. In a mixture containing 99.65 mols of NaCl to 0.35 of $CaCl_2$ the rate remains normal, while a mixture of 98.62 mols of NaCl to 1.38 of $CaCl_2$ causes a great increase in respiration.

3. The antagonism curve of NaCl vs. $CaCl_2$ is unique in that it has two maxima.

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[8] Antagonism curves with two maxima have been reported for other combinations of salts, but they seem to be somewhat different in character. Cf. **Osterhout, W. J. V.** Bot. Gaz. **48**: 98. 1909. **Brooks, M. M.** Jour. Gen. Physiol. **2**: 5. 1919. **Lipman, C. B.** Bot. Gaz. **48**: 105. 1909; Bot. Gaz. **49**: 41. 1910. **Loeb, J.** Jour. Biol. Chem. **28**: 175. 1916.